Att. to #16

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:			
Joseph R. Byrum *et al.*		Art Unit:	2833
Appln. No.:	09/206,040	Examiner:	Scott D. Priebe
Filed:	December 4, 1998	Atty. Docket:	04983.0151.US01/ 38-21 (15446)B
For:	Nucleic Acid Molecules and Other Molecules Associated with Plants		

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, **Roger C. Wiegand, Ph.D.**, make the following declaration related to matters that are within my personal knowledge:

1. I am currently employed as Vice President, Technology Acquisition, of Pharmacia Corporation's Ag Genomics Business Team and as a Fellow and Director of Technology Innovation of Cereon Genomics, LLC, a subsidiary of Pharmacia.

2. I have been with Pharmacia Corporation and its predecessor Monsanto Company and its wholly owned subsidiaries, Cereon Genomics, LLC and G.D. Searle, as a research group leader, fellow, or director, since 1985, and as a research scientist since 1981. I received my Ph.D. in Molecular Biophysics and Biochemistry from Yale University in 1976, and was a postdoctoral fellow at Yale University and Stanford University from 1976 to 1981.

3. I have over 25 years experience in molecular biology, genetics and genomics research. I am familiar with expressed sequence tag (EST) generation, cDNA library preparation, transcript profiling, nucleotide polymorphism detection, and the use of nucleic acids as hybridization probes or amplification primers.

4. I am familiar with the above-captioned application, which was filed December 4, 1998 and assigned Serial No. 09/206,040 (hereinafter the "'040 application"). The '040 application discloses an EST derived from a soybean clone designated "LIB3049-003-Q1-E1-H7", which is identified in the '040 application as SEQ ID NO: 1. The clone was isolated from a cDNA library designated "LIB3049" that was prepared from young seeds of *Glycine max* soybean cultivar Asgrow 3244.

5. A cDNA library may be generated using an oligo d(T) primed reverse transcriptase reaction. Oligo d(T) primers are designed to bind to poly A tails of mRNAs. Because mRNAs derived from the nucleus have poly A tails, and mitochondrial mRNAs do not, a cDNA library generated using an oligo d(T) primer will contain primarily cDNA clones corresponding to nuclear mRNAs, and will not normally contain cDNA clones corresponding to mitochondrial mRNA. Sequence is typically and easily screened against mitochondrial sequence. For the above reasons the existence of cDNA clones corresponding to mitochondrial mRNA would be rare. In fact, I have personally filtered SEQ ID NO: 1 against mitochondrial DNA and unequivocally say that SEQ ID NO: 1 is not similar to any known mitochondrial sequence.

6. The sequencing of cDNA clones to obtain ESTs, the generation of EST databases, and the licensing of EST databases has grown into a multi-million dollar industry. EST databases are useful tools that may be used to select clones for further research, or to compare sequences in the database with other sequences, but the nucleic acid molecules represented by the ESTs

themselves have value beyond that associated with their ESTs. Often EST database access agreements involve the physical transfer of either the clones from which the ESTs were determined or the information necessary to make nucleic acid molecules based on the sequences. For example, Clontech Laboratories, Inc. (Palo Alto, CA) sells a set of human cDNA clones called the MATCHMAKER EST library for two-hybrid analysis, and Incyte Genomics, Inc. (Palo Alto , CA) sells human, mouse, microbial and Arabidopsis cDNA clone sets and gene expression microarrays.

7. ESTs are typically used to develop molecular markers, hybridization probes, amplification primers, and to identify the presence or absence of polymorphisms.

8. cDNA clones and other nucleic acid molecules of interest may be replicated by use of the polymerase chain reaction (PCR) before their use in an experiment. PCR protocols are well-known in the molecular biology field. The generation of chimeric and/or repeated DNA may occur during PCR, but such events are not common and are readily detected and avoided. Notwithstanding that possible sequence errors, such as the misincorporation of nucleotides, may occur, nucleic acid molecules produced by PCR and containing such errors are routinely and effectively used as molecular markers, hybridization probes, amplification primers, and for identifying the presence or absence of polymorphisms. In short, the presence of such errors does not negate the usefulness of the nucleic acid.

9. The typical molecular biologist has an understanding of hybridization protocols and the polymerase chain reaction that is sufficient to take a nucleic acid molecule, such as one having SEQ ID NO: 1, and use that molecule (or, more preferably, a fragment) as a hybridization probe or amplification primer, either alone or in combination with other nucleic acid molecules.

10. There is a large body of knowledge available to molecular biologists to guide them in the use of particular nucleic acid molecules in hybridization protocols and in the polymerase chain reaction. For example, standard laboratory manuals such as *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd edition), by J. Sambrook, E.F. Fritsch, and T. Maniatis, provide extensive guidance about hybridization parameters.

11. It is routine for molecular biologists to carry out multiple experiments using different protocols so that they can determine which protocol or method is most effective with a given sequence.

12. ESTs and molecules with the EST are commonly used as molecular markers, more particularly as genetic markers, e.g. to identify a gene locus, trait locus or QTL. Molecules based on the soy EST of SEQ ID NO: 1, may be used as molecular markers even if nothing is known about them other than their sequence. For example, Southern hydridization using a nucleic acid molecule of SEQ ID NO: 1 can be used to detect a restriction fragment length polymorphism (RFLP) which is useful as a molecular marker. Molecular markers are very useful to plant breeders, and various companies, including Keygene N.V. (Wageningen, Netherlands) and AgroGene S.A., (Moissy Cramayel Cedex, France) offer molecular marker-related services for sale in connection with plant breeding.

13. Molecular biologists also know that the use of EST representative molecules, such as a nucleic acid molecule comprising the soybean EST sequence of SEQ ID NO: 1, as molecular markers may be affected by the addition of certain sequences to the nucleic acid molecule. For example, the addition of other soybean sequences, such as other soybean ESTs, to a molecule comprising the particular EST sequence of interest would, in all likelihood, prevent the efficient use of the nucleic acid molecule comprising the combined sequence as a molecular marker

because the combined molecule could hybridize to different target sequences. However, the addition of certain sequences such as a polylinker or a bacterial plasmid sequence such as pSport sequence would almost never adversely affect the use of a molecule comprising the particular EST sequence of interest as a molecular marker.

14. ESTs may also be used to generate probes for use in expression monitoring or expression profiling procedures. Soybean DNA clones, are routinely used to detect the expression levels of corresponding naturally occurring soybean nucleic acids. A nucleic acid molecule of SEQ ID NO: 1 can also certainly be used to detect expression level. Use of a nucleic acid molecule representing an EST as an expression probe is a practical use because it enables the detection of changes in expression of a particular gene.

15. I am personally aware that a nucleic acid fragment having the EST sequence represented as SEQ ID NO: 1 possesses the practical utility of being useful as a hybridization probe for expression monitoring because of the results of northern blot experiments performed by scientists under my supervision.

16. Northern blots were performed to test if a synthesized nucleic acid molecule based on SEQ ID NO: 1 would hybridize to a naturally occurring soybean nucleic acid molecule and be useful as a probe in expression profiling or for other purposes. To synthesize the nucleic acid molecule eight overlapping 60-80-mer oligonucleotides were prepared to cover the 469 bases of SEQ ID NO: 1. The oligonucleotides copied segments of SEQ ID NO: 1 except that random nucleotides were inserted into the "N" labeled positions of SEQ ID NO: 1 at positions 2, 3, 437 and 455. PCR was used to amplify a synthetic polynucleotide population using the set of eight overlapping oligonucleotides as a template. Direct sequencing of the synthetic polynucleotide population did not produce definitive sequence information. Accordingly, five random

polynucleotides from the population were cloned and sequenced to verify trueness of copy. See Exhibit A. Analysis of the cloned sequences showed that the synthesized polynucleotides had a consensus sequence identical to SEQ ID NO: 1. Among the five sequenced synthetic polynucleotides deviations from SEQ ID NO: 1 included single nucleotide mutations (not more than 1 per molecule) and single nucleotide insertions (from 1 to 6 per molecule). The relationship of these sample sequences to SEQ ID NO: 1 as well as identity of the consensus sequence of the synthetic probe with SEQ ID NO: 1 suggests that the synthetic probe is a true enough copy of SEQ ID NO: 1 for use as a probe to demonstrate the utility of nucleic acid molecules characterized by SEQ ID NO: 1.

17. Using a standard northern blot protocol the synthesized nucleic acid molecule was used as a probe against two distinct preparations of *Glycine max* mRNAs having poly A tails - one derived from total plant (sprout) tissue, and the other derived from adult leaf tissue. The synthesized molecule did hybridize to the soy mRNAs in both blots, yielding a prominent band at approximately 1.6 kilobases. A northern blot showing this hybridization to the synthesized molecule is attached as Exhibit B.

18. A northern blot was also performed using a probe comprising the SEQ ID NO: 1 region from the plasmid that carries clone LIB3049-003-Q1-E1-H7. The probe derived from the plasmid also hybridized to the soy mRNAs in both blots, yielding a similar prominent band at approximately 1.6 kilobases.

19. The results of the northern blots indicate that a nucleic acid molecule having the sequence of SEQ ID NO: 1 can be synthesized and successfully used as a hybridization probe, and that such a molecule will hybridize to a naturally occurring soybean nucleic acid molecule.

Accordingly, a nucleic acid molecule having SEQ ID NO: 1 is useful as a hybridization probe for expression profiling or other purposes.

20. Nucleic acid molecules comprising ESTs or other nucleic acid molecules which are capable of detecting genetic polymorphisms are useful in plant breeding. The detection of polymorphisms is useful for genetic analysis and the development of genetic maps to tag important agronomic traits such as tolerance to abiotic stress and pathogen resistance. The EST represented by SEQ ID NO: 1 was generated from a *Glycine max* cDNA clone. It was therefore probable that a molecule comprising SEQ ID NO: 1 would be capable of detecting a polymorphism in a population of plants derived from a cross such as a cross between *Glycine max* and *Glycine soja*. The use of nucleic acid molecules representing ESTs to detect polymorphisms is a practical utility because it enables a plant breeder to determine the distribution of parental genetic material in the progeny of a cross.

21. I believe that a nucleic acid molecule comprising the EST of SEQ ID NO: 1 possesses the practical utility of being useful for detecting polymorphisms because scientists under my supervision performed Southern blots to test if a synthetic nucleic acid molecule based on SEQ ID NO: 1 would detect polymorphisms. It did.

22. Two probes were prepared for the Southern blots: a synthesized nucleic acid molecule based on overlapping oligomers matching SEQ ID NO: 1; and a probe derived from the plasmid that carries clone LIB3049-003-Q1-E1-H7, from which SEQ ID NO: 1 was determined. A standard Southern blot protocol was used to probe digested soybean chromosomal DNA derived from *Glycine max* or *Glycine soja* plant tissue. In four of twenty restriction digests examined a length polymorphism was evident between *Glycine max* and *Glycine soja,* indicating the presence of four restriction fragment length polymorphisms between the *Glycine max* and

Glycine soja plants. A Southern blot showing this identification of length polymorphisms is attached as Exhibit C.

23. The results of the Southern blots indicate that a nucleic acid molecule having the sequence of SEQ ID NO: 1 can be synthesized and successfully used to detect polymorphisms in soybean chromosomal DNA. Accordingly, a nucleic acid molecule having the sequence of SEQ ID NO: 1 is useful for detecting polymorphisms in order to develop a genetic map, determining if a plant carries the gene for a particular trait, determining the copy number of a particular gene in a plant, or for other purposes.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Roger C. Wiegand

Roger C. Wiegand, ~~Ph.D~~.

Executed on August 17, 2000.

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Molecule 1-1   A:CCAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 2-1   ATGAATCCTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 2-2   ATGAATCCTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 3-1   A:GTAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 3-2   A:GTAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 4-1   A:TAAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCAGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 5-1   A:CCAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Molecule 5-2   A:CCAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC

Base number    1
SEQ ID NO: 1   A:NNAT:CTTCAATGTATCATCCACAGATATAGCAGATTGTCCCTGGCCAGTGATCCTATCGAGCTCTTTGATGAGATAAACGCGATTC
Mut/Ins         i    i                                        m


Molecule 1-1   TCTGCCGTCCTGCCACAGCCTTCCAG:CGACGTT:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 2-1   TCTGC:GTCCTGCCACAGCCTTCCAG:CGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 2-2   TCTGC:GTCCTGCCACAGCCTTCCAG:CGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 3-1   TCTGC:GTCCTGCCACAGCCTTCCAAGCGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 3-2   TCTGC:GTCCTGCCACAGCCTTCCAAGCGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 4-1   TCTGC:GTCCTGCCACAGCCTTCCAG:CGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 5-1   TCTGC:GTCCTGCCACTGCCTTCCAGCCGACGTAAGATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Molecule 5-2   TCTGC:GTCCTGCCACTGCCTTCCAGCCGACGTAAGATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG

Base number    90
SEQ ID NO: 1   TCTGC:GTCCTGCCACAGCCTTCCAG:CGACGTA:GATAGAAGCGAAAGTGGCGAACATGGAGATGAGGAGCGCGGGAATGCGGGATCG
Mut/Ins             i          m         mi       mi


Molecule 1-1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 2-1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 2-2   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 3-1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 3-2   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 4-1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATCCTGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 5-1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATCCTGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Molecule 5-2   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATCCTGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG

Base number    179
SEQ ID NO: 1   ATTATTGCCCATACTGGAGAGTCTGTGACTCGATCCCCTGATC:TGCATTTTTGAAATCAATGCAAATGCAATCTTATGAACGATTATG
Mut/Ins                                                 i
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Molecule 1-1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCTG
Molecule 2-1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCGG
Molecule 2-2   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCGG
Molecule 3-1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCTG
Molecule 3-2   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCTG
Molecule 4-1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCTG
Molecule 5-1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGCCTACTTTTCTG
Molecule 5-2   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGCCTACTTTTCTG

Base number  268
SEQ ID NO: 1   ATATTGTATATGTAGATGCAGAGAGAATTGGATTAGAATTCGATAGATGCAAAGCAAAGTCTTTGGTGCTGCGGTTGC:TACTTTTCTG
Mut/Ins                                                                                  i        m


Molecule 1-1   GTCAA:GAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 2-1   GTCAAGGAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 2-2   GTCAAGGAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 3-1   GTCAA:GAAAGTCACCCAAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 3-2   GTCAA:GAAAGTCACCCAAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 4-1   GTCAA:GAAAGTCACCAAAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 5-1   GTCAA:GAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCCCTTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Molecule 5-2   GTCAA:GAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCCCTTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG

Base number  357
SEQ ID NO: 1   GTCAA:GAAAGTCACC:AAGAATCCACTCTCTTTCCCCCCCCC:TTCTGTCGTCTTTCGCAGCCGTACGGACGCGTGGGTCGACCCGGG
Mut/Ins             i          i                        i


Molecule 1-1   AATTCCTGACCGCTACCTGG:ACGCTGCAAGTTAA
Molecule 2-1   AATTCCTGACCGGTACCTGG:CCGCTGCAAGTTAA
Molecule 2-2   AATTCCTGACCGGTACCTGG:CCGCTGCAAGTTAA
Molecule 3-1   AATTCCTGACCGCTACCTGG:CCGCTGCAAGTTAA
Molecule 3-2   AATTCCTGACCGCTACCTGG:CCGCTGCAAGTTAA
Molecule 4-1   AATTCCTGACCGATACCTGG:GCGCTGCAAGTTAA
Molecule 5-1   AATTCCTGACCGTTACCTGGGCCGCTGCAAGTTAA
Molecule 5-2   AATTCCTGACCGTTACCTGGGCCGCTGCAAGTTAA

Base number  446
SEQ ID NO: 1   AATTCCTGACCGNTACCTGG:NCGCTGCAAGTTAA
Mut/Ins                         i
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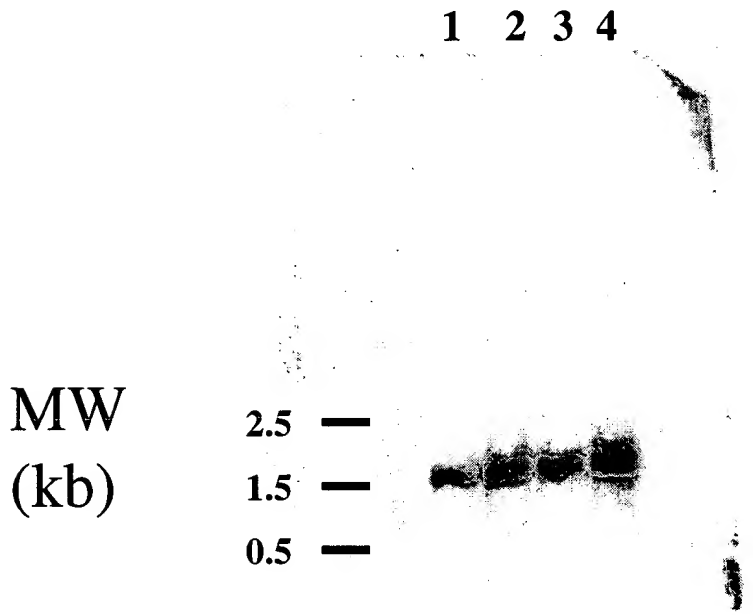
Exhibit B

Northern Blot using seq ID#1 as probe against tissue from G max strain A3244



1. 0.5 µg mRNA
2. 1.5 µg mRNA

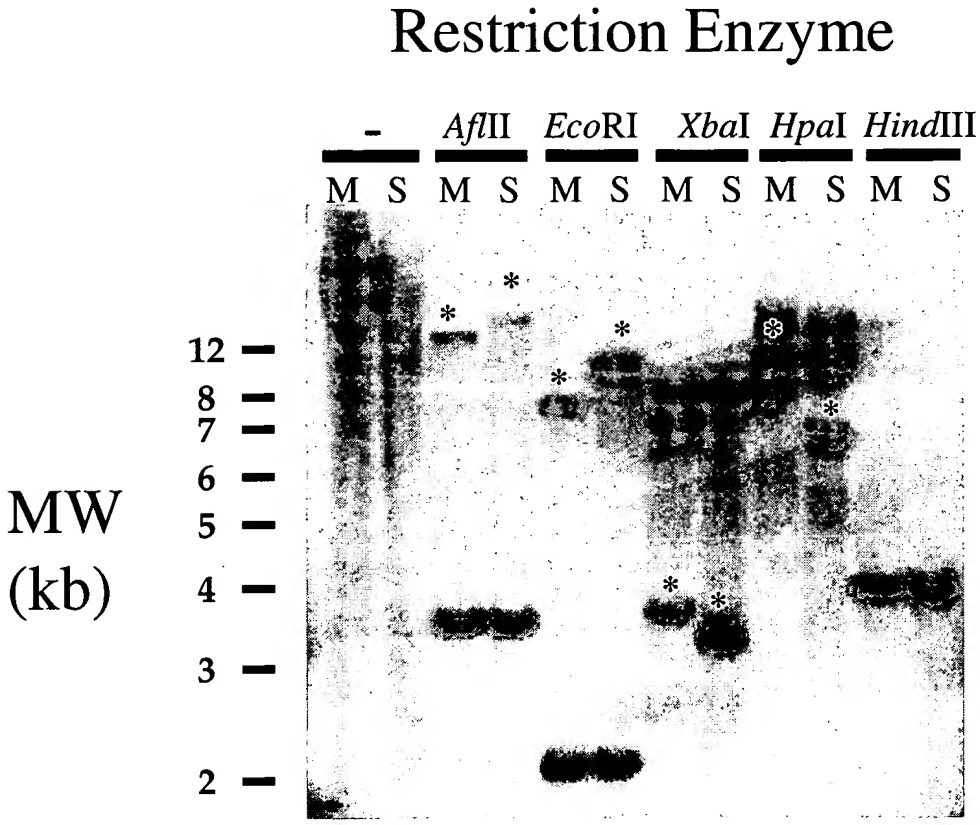
} Adult Plant Leaf

3. 1.0 µg mRNA
4. 3.0 µg mRNA

} Sprouting Seeds

Exhibit C

Southern Blot to detect Restriction FragmentLength Polymorphisms (RFLP) between G max and G soja using Seq ID#1 as probe



M – Glycine max strain A3244
S – Glycine soja strain Pic

*** - denotes a fragment which is polymorphic in length between G max and G soja.**

Pretransformed MATCHMAKER EST Library

A universal library for studying protein interactions

- **Obtain more interactors in a single screen**
- **More than 250,000 human ESTs**
- **Pretransformed for fast results**
- **Proven, reliable system**

Perform the most complete two-hybrid screen in the shortest time with the **Human Pretransformed MATCHMAKER EST cDNA Library**†—the first normalized, universal expressed sequence tag (EST) cDNA library for two-hybrid analysis. It combines the stringent selection of our AH109 yeast strain with a reliable EST library encompassing 28 human tissues and organs from various developmental and disease states. With the library's proven reliability and broad tissue coverage, you can focus your efforts on positive protein interactions after just one library screen.

Our pretransformed EST library provides the greatest chance of finding rare or novel protein interactions. It contains sequences from a comprehensive collection of human tissues, and the abundance of each sequence is normalized so that rarely expressed genes are present at the same levels as those normally expressed at higher levels. In addition, each sequence is represented in all three reading frames. Because the library is pretransformed, you can identify putative interacting proteins in less than two weeks.

A carefully constructed, universal library

The ESTs included in this library were initially collected and sequenced by the IMAGE Consortium (Integrated Molecular Analysis of Genomes and their Expression)—a publicly available EST collection (1). The EST library consists of 251,520 ESTs from this collection cloned into the GAL4 activation domain vector pACT2 (2).

Figure 1 shows how we constructed the EST Library. First, we amplified cDNA inserts in all three reading frames and mixed pools of fragments with linearized pACT2 DNA. The EST insert integrated into the pACT2 vector multiple cloning site by homologous recombination, which is highly efficient in yeast (3). Figure 2 shows a representative pool of 8 ESTs in *E. coli* that successfully integrated into yeast by homologous recombination.

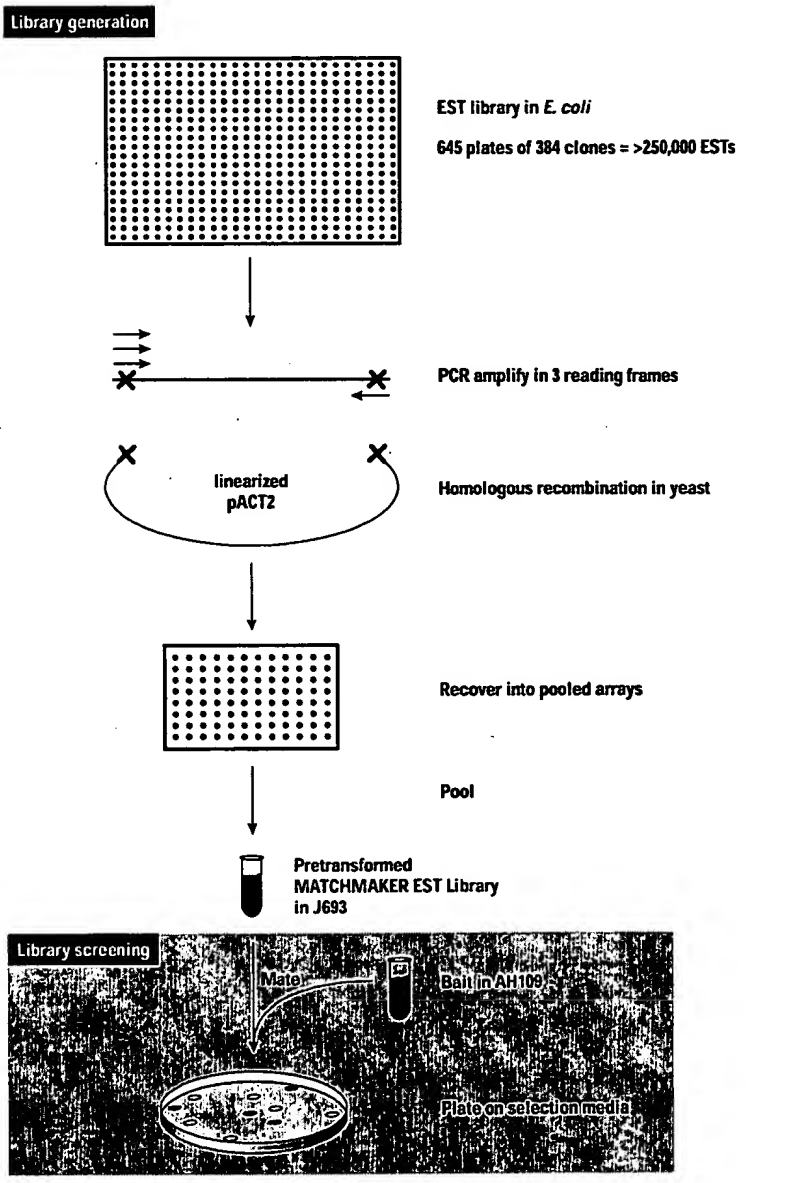


Figure 1. The Pretransformed MATCHMAKER Human EST cDNA Library is a carefully constructed library composed of more than 250,000 pooled human ESTs. Screening the library requires only one mating, then plating on selection media.

Pretransformed for fast results

Our EST library is pretransformed in yeast strain J693. By eliminating the amplification and library-scale transformation steps, your library screening process is reduced to only one to two weeks. You simply mate the library strain with AH109 that you have transformed with a DNA-binding domain vector containing your bait construct. The next day, plate the mating mixture on growth medium that selects for two-hybrid interactions.

MATCHMAKER EST Library...continued

Figure 2. Homologous recombination is highly efficient in yeast. Lane 1: An original pool of 8 EST inserts in *E. coli*. Lane 2: The same inserts after homologous recombination in yeast strain J693. M = Marker.

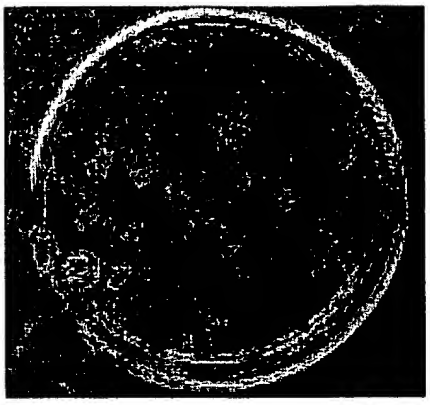


Figure 3. Positive clones from a two-hybrid experiment using AH109. 1.2 million diploid colonies were screened by mating the EST library with the pAS2-1-IAP1 bait in AH109. After 6 days of incubation, the resulting 20 *Ade2*$^+$, *His3*$^+$ colonies were assayed for α-galactosidase activity. Four were positive. One additional *Ade2*$^+$ *His3*$^+$ α-Gal$^+$ colony grew up after 12 days of incubation (data not shown).

AH109, which we include with the library, is the most advanced yeast strain for two-hybrid analysis. It contains *ADE2*, *HIS3*, *lacZ*, and *MEL1* reporter genes, each of which uses a distinct GAL4-responsive promoter (4). These distinct promoter elements virtually eliminate false positives for the most reliable and accurate two-hybrid screen.

A proven system

With the Pretransformed MATCHMAKER EST library, you can obtain more positive clones than by conventional library screening. In one experiment, Hua *et al.* (2) screened for proteins that interact with the human apoptosis inhibitor IAP-1 by screening either a pool of 3 human tissues or the EST library (Table I; 2). After screening 36 million clones from the EST library, they identified one known and nine novel interactors—six more novel interactors than they found by screening the pool of three tissues.

The results were proportionally higher when we performed the screen by mating the library strain to AH109 that we transformed with DNA-binding domain vector containing IAP-1. From this mating, we identified five novel interactions from only one million clones (Figure 3).

A complete kit for two-hybrid screening

Each order includes 5 x 1–ml aliquots, each of which is sufficient for a complete library screening of >2 x 10^6 independent clones. The library is provided with pretransformed control strains to test for a positive two-hybrid interaction, AH109, a Complete User Manual, and a Vector Information Packet. CLONTECH also offers a complete line of yeast media and dropout supplements—see **matchmaker.clontech.com** or the current catalog for details.

Table I: Comparison of two-hybrid library screening

Library	Conventional*	EST	EST
Technique	Transformation in Y190	Transformation in J693	Mating with AH109
Total transformants (millions)	69	36	1
Positive clones	4	10	5
Known partner	TRAF2	TRAF2	0
New interactions	3	9	5

* Combination of three pooled libraries.

Product	Size	Cat. #
Human Pretransformed MATCHMAKER EST cDNA Library	5 x 1 ml	HY4100AH

NEW!

Components

- **Pretransformed Library in Yeast Strain J693**
- **Yeast Strain AH109** (MATa)
- **Yeast Strain J693** (MATα)
- **pAS2-1-IAP1 in AH109**
- **pACT2-TRAF2 in J693**
- **Complete User Manual** (PT3495-1)
- **Library Vector Information Packet** (PT3022-5)

Yeast strain J693 genotype: MATα, *ura3-52, his3-Δ200, ade2, trp1, leu2, gal4Δ gal80Δ, URA3::GAL-lacZ, cyh^{r}2, LYS2::GAL-HIS3, MEL1.*

Yeast strain AH109 genotype: MATa, *trp-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ, MEL1.*

Related Products

- **MATCHMAKER Two-Hybrid System 3** (#K1612-1)
- **pGBKT7 DNA-BD Vector** (#K1612-A)
- **pBridge™ Three-Hybrid Vector** (#6184-1)
- **YEASTMAKER™ Carrier DNA** (K1606-A)
- **YEASTMAKER™ Yeast Transformation Kit** (#K1606-1)
- **X-α-Gal** (#8061-1)
- **AH109 Competent Yeast Cells** (#C5001-1)
- **Yeast Media & Dropout Supplements** (many)

References

1. Hillier, L., *et al.* (1996) *Genome Res.* 6:807–828.
2. Hua, S., *et al.* (1998) *Gene* 215:143–152.
3. Hua, S., *et al.* (1997) *Plasmid* 38:91–96.
4. MATCHMAKER Two-Hybrid System 3 (January 1999) *CLONTECHniques* XIV(1):12–14.

[1] U.S. Patent #5,989,872.

Notice to Purchaser

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IncyteGenomics For Life. Reagents & Services

Clone Sets for GEM Microarrays

For further *in vitro* analysis, you can purchase clones for all elements on our GEM™ microarrays. Clones can be purchased individually or as a set. We ship individual clones as agar stabs. We can ship clone sets in either 384- or 96-well plates.

Search Gene Lists
Search for your genes of interest to see how many related ESTs are on GEM microarrays

GEM Clone Lists

GEM FASTA Files

Full Bioinformatic Report

A full bioinformatic report is provided with each clone set, including clone ID, plate and well location, GenBank accession number, UniGene clustering information (if applicable), and gene annotation (where available).

Clones for the Human UniGEM™ V 2.0 Microarray

More than 9,100 sequence-verified Incyte clones mapped to NCBI's UniGene database. Use of these sequence-verified Incyte clones does not obligate the researcher to publish experiment results.

Cat #	Item	Price	Order
	GEM Microarrays		
GEM-5113	Human UniGEM V 2.0 Clone Retrieval (per clone)	**$ 45**	add to cart
GEM-5114	Human UniGEM V 2.0 Clone Set	**quote**	add to cart
GEM-5101	Human UniGEM V 1.0 Clone Retrieval (per clone)	**$ 45**	add to cart

Clones for the Mouse GEM™ 1 Microarray

Over 8,700 Incyte-verified IMAGE Consortium clones mapped to NCBI's UniGene database. Use of IMAGE Consortium clones obligates the researcher to certain publishing requirements. Contact us for more information at clones@stl.incyte.com.

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Cat #	Item	Price	Order
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GEM-5201	Mouse GEM 1 Clone Retrieval (per clone)	**$ 45**	add to cart
GEM-5210	Mouse GEM 1 Clone Set	**quote**	add to cart

Clones for the Arabidopsis GEM™ 1 Microarray

Over 7,900 Incyte-verified Public Domain and Incyte clones mapped to GenBank plant databases. Use of these clones do not obligate the researcher to publish experiment results.

Cat #	Item	Price	Order
	GEM Microarrays		
ARB-5351	Arabidopsis GEM 1 Clone Retrieval (per clone)	**$ 45**	add to cart
ARB-5352	Arabidopsis GEM 1 Clone Set	**quote**	add to cart

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Keygene MMS offers a wide range of molecular marker services for use in plant breeding. The major applications are:

- Genetic mapping
- Marker Assisted Back-Crossing (MABC)
- Genetic distance analyses
- Purity analyses, identity confirmation, genetic stability confirmation, etc.
- Identification of AFLP® markers for indirect selection of monogenic traits
- The identification and fine mapping of QTL - Reversed QTL Mapping
- The removal of linkage drag
- Marker database applications
- The identification of individuals who are homozygous for a transgene
- The design of simple assays for indirect selection
- The identification and exploitation of genetic diversity

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Molecular Markers

AGROGENE provides to its customers four different techniques

➤ **RFLP (Restricted Fragment Length Polymorphism)**
This technique is based on the use of Southern blots and of probes, not patented.

➤ **PCR (Polymerase Chain Reaction)**
AGROGENE has a licence from Perkin-Elmer Corporation and provides services based on PCR to customers with the mention "This test is performed persuant to licensing with the Perkin-Elmer Corporation and patent rights owned by F. Hoffman La Roche Ltd".

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Based on PCR, developed and patented by Keygene n. v. AGROGENE has taken a licence from Keygene to provide services based on AFLP™ to customers.

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